Stress and Acid-Base Balance in Chickens[1]

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ABSTRACT Two trials were conducted to study the effects of continuous infusion of adrenocorticotropic hormone (ACTH) on acid-base balance in broiler chickens. Osmotic pumps delivered 8 IU of ACTH in saline/kg of BW/d for 7 d or the same saline volume as used in ACTH at 1 μL/h for 7 d. Blood samples were taken on d 0 (baseline values) and on d 4, 7, and 14 after onset of the infusions. The ACTH treatment increased the hematocrit, partial pressure of CO_2, anion gap, corticosterone, mean corpuscular hemoglobin concentration, the blood concentrations of hemoglobin and HCO_3^-, and reduced the partial pressure of O_2, plasma concentrations of Na^+, K^+, and Cl^-. Blood pH values and plasma concentrations of Ca^{2+} were unaffected by ACTH treatment. The ACTH infusion also resulted in a significant increase in plasma glucose, cholesterone, high-density lipoprotein, and triglyceride. There were no differences in any of the blood constituents measured from control groups. Results indicate that infusion of ACTH resulted in changes in plasma acid-base status along with changes in other blood metabolic variables. However, the ACTH treatment did not prevent homeostatic regulation of acid-base balance, as indicated by constant blood pH. There was, however, an increased need for O_2 to support gluconeogenic energy production; the birds responded by increased erythropoiesis. This adaptive response provided greater numbers of erythrocytes and thus a higher amount of circulating hemoglobin to deliver O_2 for metabolism.

Key words: acid-base balance, stress, adrenocorticotropic hormone, broiler

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INTRODUCTION

Stress describes an animal's defense mechanisms, and thus a stress stimulus (stressor) is any situation that elicits adaptive responses (Selye, 1936). Any combination of conditions in which birds live may act as a stressor. For instance, climatic (extreme heat and cold, high humidity), environmental (poor ventilation, bright light, wet litter), nutritional (shortages of nutrients, feed intake problems), physical (catching, immobilization, injection, transport), social (overcrowding, poor body weight uniformity), physiological (rapid growth, process of maturing sexually), psychological (fear, harsh caretakers, noise), and pathological (exposure to infectious agents) conditions may induce a state of stress in an animal, thereby reducing welfare and performance (Freeman, 1987; Scheele, 1997; Feltenstein et al., 2003; Cheng and Muir, 2004, Corzo et al., 2005). When the threshold level of stress is crossed, it results in stress syndromes that are classified into neurogenic (stage of alarm reaction), endocrine mediated (stage of resistance of adaptation), and metabolic depletion (stage of exhaustion). Adrenocorticotropic hormone (**ACTH**) stimulates the adrenal cortex, which in turn releases corticosteroids, primarily corticosterone (**CS**) in birds. Corticosterone produces many symptoms associated with long-term stress such as cardiovascular disease (arteriosclerosis, ascites) and modification of immunological functions (Grandin, 1998). Poultry producers are trying to control these factors so that the birds can maintain normal physiological functions and produce meat or eggs at maximum rates.

Acid-base balance of any animal is influenced by a range of functional and environmental stressors. The pattern of change depends upon the effects of these stressors on the condition and rate of metabolism, respiration, and the mechanism of H^+ equivalent exchange. In a feedback reaction, acid-base factors, especially pH, affect metabolic processes and are important to the overall rate of energy turnover. Physiological stress is associated with changes in acid-base status in a number of species (Aguilera-Tejero et al., 2000; Jochem, 2001; Sandercock et al., 2001; Derjant-Li, et al., 2002; Parker et al., 2003; Borges et al., 2003, 2004; Yalcin et al., 2004). However, interpretation of acid-base balance disorders especially during stress is complicated because many relevant variables change simultaneously and in many instances in opposite directions depending on species and type of stressor.

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Until recently, a model to study physiological adaptive responses of fowl subjected to stressful conditions has been lacking. Puvadolpirod and Thaxton (2000a,b,c,d) presented such a model that involves continuous infusion of ACTH via mini-osmotic pumps. Forty-two adaptive responses to ACTH were reported, including elevated circulating levels of CS, glucose (**GLU**), cholesterol (**CHOL**), triglycerides (**TRI**), and lipoproteins, as well as increased heterophil:lymphocyte ratio. Odihambo et al. (2006) extended this model to adult hens and found that, in addition to the responses found in juvenile birds, adult hens ceased reproduction caused by atrophy of mature ovarian follicles.

Puvadolpirod and Thaxton (2000a,b,c,d) did not document the effects of ACTH infusion on blood gases and electrolytes. However, the effects of stress on electrolytes in birds is well known relative to heat stressors. Hyperthermia causes increased body temperature and respiratory rate, along with a decrease in blood partial pressure of carbon dioxide (**pCO_2**). The decrease in pCO_2 results in changes in acid-base balance leading to respiratory alkalosis (Raup and Bottje, 1990; Macari et al., 1994). Richards (1970) was the first to show that thermal panting in birds can lead to respiratory alkalosis. Kohne and Jones (1975a) reported acute hyperthermia in turkey hens produced profound respiratory alkalosis, whereas chronic hyperthermia had no effect (Kohne and Jones, 1975b). Siegel et al. (1974) reported heat stress in broilers reduced blood pCO_2 and HCO_3^-. Recent work has expanded the understanding of the essential role dietary electrolytes exert on acid-base balance and the resistance to respiratory alkalosis caused by hyperthermia (Borges et al., 2003; Ahmed et al., 2005).

The purpose of the present work was to expand the stress model of Puvadolpirod and Thaxton (2000a,b,c,d) to include physiology of acid-base balance. The rationale was to infuse ACTH osmotically and investigate changes in acid-base balance, as well as metabolic parameters and CS during the stress period.

MATERIALS AND METHODS

Bird Husbandry

Three-hundred sixty male chicks Ross (Aviagen Inc., Huntsville, AL) × Cobb (Cobb Vantress, Siloam Springs, AR) were obtained from a commercial hatchery and randomly placed into 6 environmentally controlled chambers (60 chicks/chamber). Each chamber was divided into 2 pens each having 30 chicks (2 replicates of 30 chicks in each chamber). Chicks were vaccinated for Mareks, Newcastle, and infectious bronchitis at the hatchery. Each chamber contained fresh pine shavings, tube feeders, and a nipple watering system with 7 nipples. A 3-phase feeding program (starter from d 1 to 15, grower from d 16 to 28, and finisher from d 29 to 42) was provided that consisted of corn-soybean meal-based diets formulated to meet or exceed NRC (1994) nutrient recommendations. Starter feed was provided as crumbles, and subsequent feeds were fed as whole pellets. Feed and water were offered ad libitum, and lighting was continuous at approximately 10 lx throughout the study. Ambient temperature was maintained at 33°C at the start of experimentation and reduced as the birds progressed in age to ensure comfort with a final temperature of 21°C at 35 d and thereafter.

Treatment

Twelve birds (6 birds in each of the 2 pens) in each of the 6 chambers served as treated birds, and 48 birds in each chamber were extra birds. Three chambers were randomly selected for each treatment (ACTH, saline). The 48 extra birds in each chamber were used to establish baseline physiological parameters and to minimize social interactions. On d 35, all birds in each chamber were weighed as a group. Then, the 12 birds in each chamber that had been surgically implanted with a mini-osmotic pump were marked with spray paint for visual identification. These pumps were loaded with ACTH or saline. Pumps loaded with ACTH (Sigma-Aldrich Fine Chemicals, St. Louis, MO) delivered 8 IU of ACTH/kg of BW/d for 7 consecutive days. Control (**CON**) birds received pumps that delivered saline at a volume equivalent to that of ACTH-treated birds for 7 consecutive days. Delivery rate of all pumps was 1 μL/h. Puvadolpirod and Thaxton (2000a) have described implantation of the pumps in detail.

Blood Collection and Chemistry Analysis

Four extra birds (2 from each pen replicate within each chamber) were selected at random and bled immediately before the pumps were implanted. After blood collection, these birds were removed from the study. These blood samples were analyzed to provide baseline physiological values. Then on d 4, 7, and 14 after pumps were implanted, 4 birds from each chamber (2 birds from each pen replication) that possessed pumps were bled and then removed from the experiment.

Blood samples were collected by cardiac stab into heparinized (50 IU/mL^{-1}) monovette syringes. Each bird was placed on its right side, and the needle was inserted between the third and fourth true ribs. We always made sure that the needle pierced the left ventricle, judging from the color of the blood, which was bright red unlike dark red from the right ventricle. All bleedings were completed within 45 s after birds were caught. Blood was drawn directly from the syringes into a blood gas/electrolyte analyzer (ABL-77, Radiometer America, Westlake, OH) for immediate analysis of pCO_2, partial pressure of oxygen (**pO_2**), pH, hematocrit (**Hct**), hemoglobin (**Hb**), and electrolytes (Na^+, K^+, Ca^{2+}, HCO_3^-, and Cl^-). The pH, pCO_2, pO_2, and HCO_3^- values were corrected to reflect body temperature of 41.5 C (Burnett and Noonan, 1974; Fedde, 1986). The mean corpuscular hemoglobin concentration (**McHc**) was calculated using the standard formula. The needle mounted on each monovette syringe

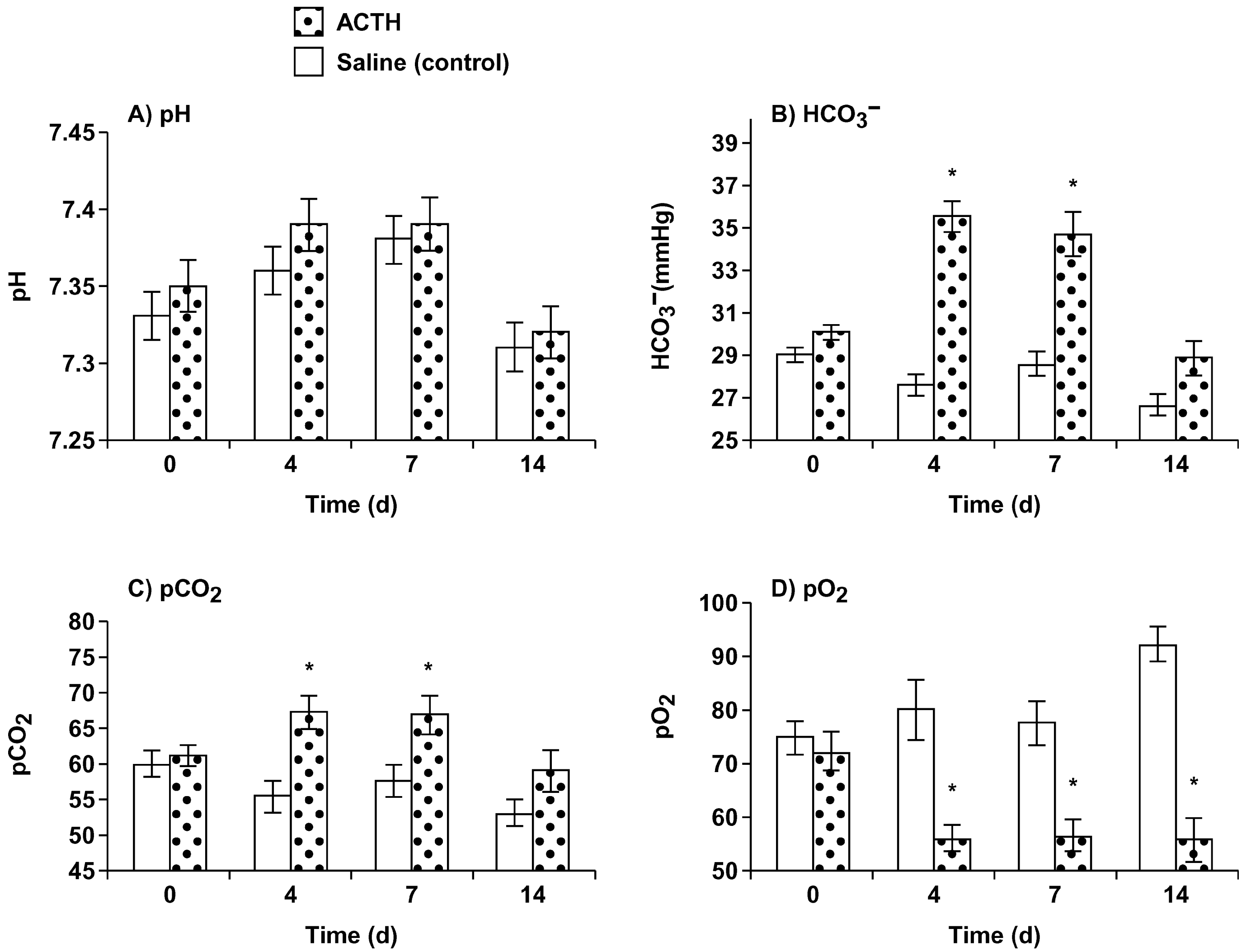

Figure 1. Circulating levels of pH (A), HCO_3^- (B), pCO_2 (C), and pO_2 (D) in broilers after adrenocorticotropic hormone (ACTH) infusion. Data are presented as the mean ± SEM. Time measurement corresponds to time of pump placement. *Differs ($P < 0.05$) from corresponding control mean.

was then removed, a cap was placed over the needle port, and the syringes containing the blood samples were plunged into ice. After all birds were bled, the iced samples were transferred to the laboratory, centrifuged at 4,000 × *g* for 20 min, and the packed blood cells were expelled from the syringes. The plunger on each monovette was broken off, and the syringe served as a storage vial for the remaining plasma. This procedure insured that the plasma samples were never exposed to ambient air to prevent air contamination. Plasma samples were stored at –20°C for later chemical analysis. Plasma samples were removed from the freezer, thawed, and each sample was analyzed for CS, CHOL, GLU, TRI, and high-density lipoprotein (**HDL**). Concentrations of all plasma chemical constituents, with the exception of CS, were determined using an auto analyzer (Ektachem model DT 60). This analyzer employs enzymatic procedures that have been described by Elliott (1984). Plasma CS was measured using universal microplate spectrophotometer (BioTek Instrument Inc. Winooki, VT) with ELISA (ELISA-CS Kit, Assay Designs Inc., Ann Arbor, MI) reagent assay test kits from Assay designs (Assay Designs Inc.) according to manufacturer's instruction.

Statistical Analysis

Each trial was analyzed by a randomized complete block design with each day of sample collection as a block. Data were pooled for ease of presentation and subjected to the GLM procedure of the Statistical Analysis System (SAS Institute, 1990). Each treatment mean ± SEM represents 6 replicated chambers (i.e., 3 chambers per trial). Differences between treatment means were assessed by LSD, and statements of significance are based on $P < 0.05$.

RESULTS

The effects of continuous ACTH infusion on pH and blood gases are presented in Figure 1. Blood pH values were unaffected by ACTH treatment on any of the days of measurement (Figure 1A). ACTH infusion increased the blood concentrations of HCO_3^- and pCO_2 on d 4 and

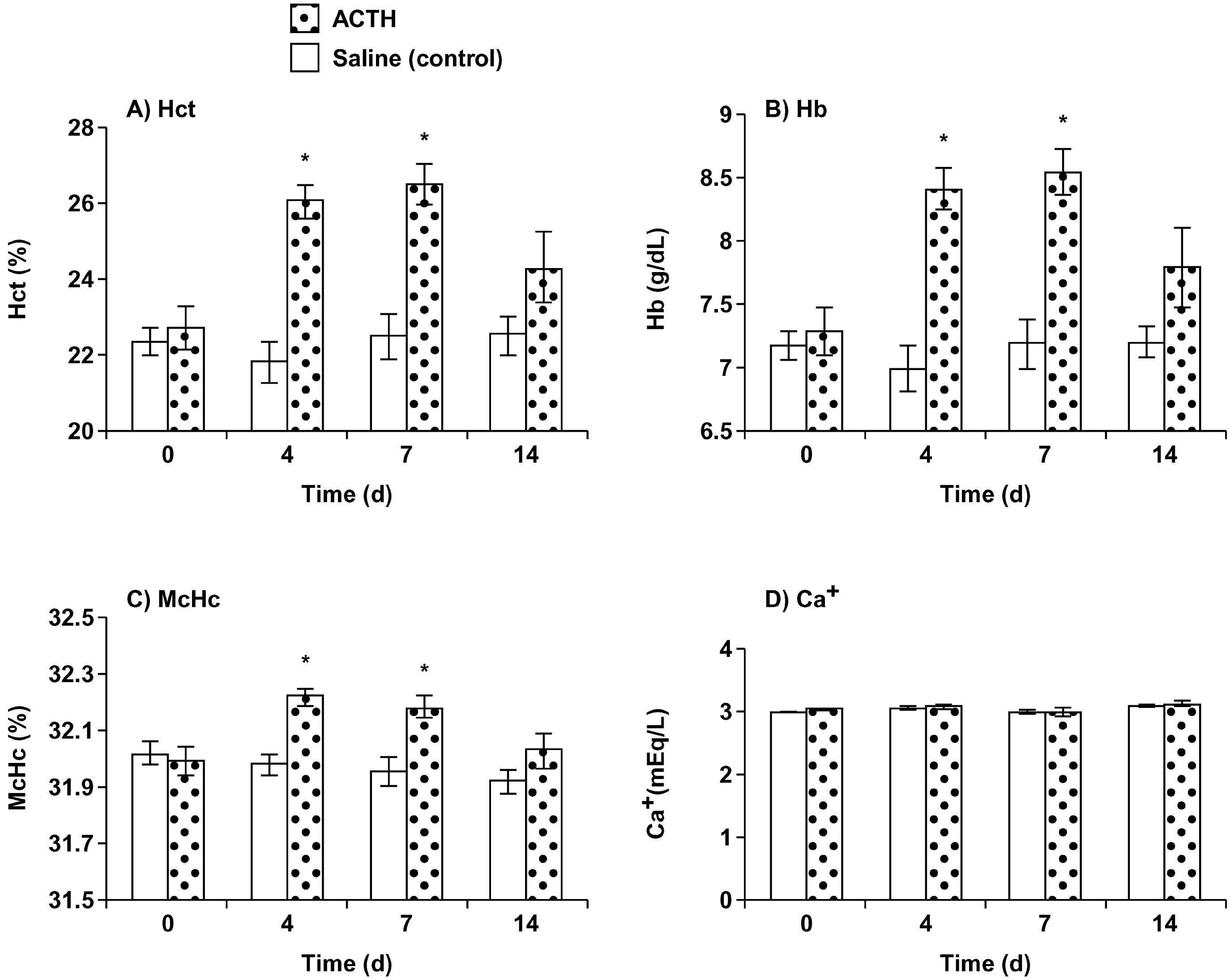

Figure 2. Hematocrit (Hct, A), circulating levels of hemoglobin (cHb, B), mean corpuscular hemoglobin concentration (McHc, C), and (Ca^{2+}, D) in broilers after adrenocorticotropic hormone (ACTH) infusion. Data are presented as the mean ± SEM. Time measurements correspond to time of pump placement. *Differs ($P < 0.05$) from corresponding control mean.

7, and reduced pO_2 on d 4, 7, and 14. Hematocrit and McHc values along with blood concentrations of Hb were increased in ACTH-treated birds, compared with the CON on d 4 and 7, but Ca^{2+} levels were unaffected (Figure 2). Post-ACTH infusion, the blood concentration of Na^+ was decreased on d 4 and 7 but elevated on d 14. Blood concentrations of K^+ and Cl^- were decreased on d 4 and 7, whereas that of anion gap decreased on d 4, 7, and 14 (Figure 3). The ACTH infusion also resulted in a significant increase in plasma concentrations of GLU, CHOL, HDL, and TRI (Figure 4). There were no differences in any of the blood constituents measured from CON groups. Changes in plasma concentrations of CS are illustrated in Figure 5. The average concentration of CS in baseline and CON birds on all 3 d of measurement was 11.10 ng/mL. The CS concentrations in the ACTH-treated birds were elevated by approximately 18-, 26-, and 7-fold on d 4, 7, and 14, respectively.

DISCUSSION

The present results indicate that continuous delivery of ACTH at 8 IU/kg of BW/d for 7 d at a rate of 1 uL/h via a mini-osmotic pump stimulates CS release that induces physiological stress in broiler chickens. Previous reports support this conclusion (Latour et al., 1996; Puvadolpirod and Thaxton, 2000a,b,c,d). In the present study, continuous delivery of ACTH increased plasma CS, GLU, CHOL, HDL, and TRI during the postimplantation period. Evaluations of plasma levels of CS, GLU, CHOL, and TRI are in agreement with metabolic changes associated with stress in chickens (Mickey et al., 1996; Puvadolpirod and Thaxton, 2000a,b). Increased circulating glucocorticoid levels are known to result in gluconeogenesis with a resultant increase in circulating concentrations of GLU, CHOL, and heterophil:lymphocyte ratio (Siegel, 1961, 1971). Reasons for increases in GLU due to stress are

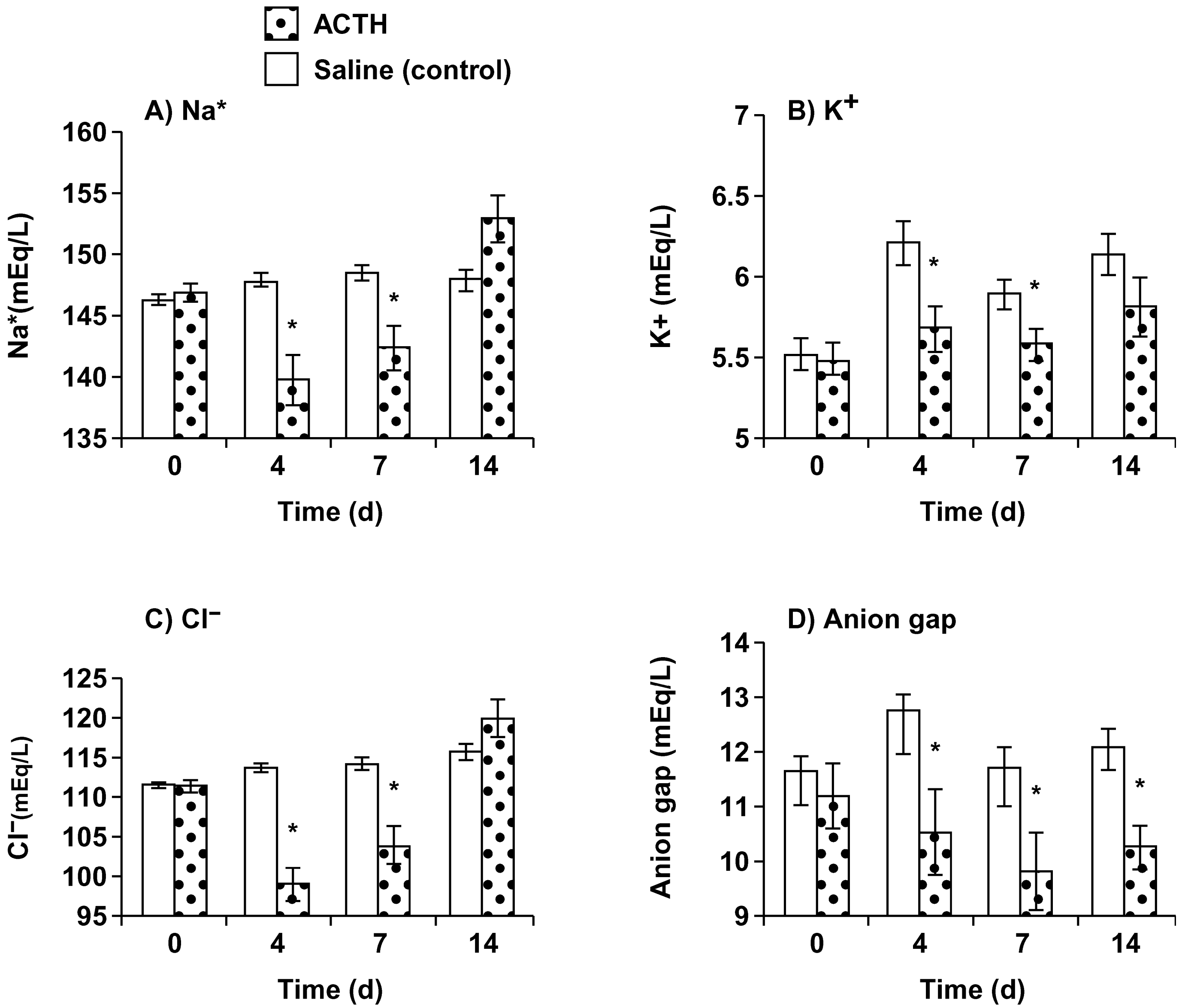

Figure 3. Circulating levels of Na^+ (A), K^+ (B), Cl^- (C), and calculated anion gap (D) in broilers after adrenocorticotropic hormone (ACTH) infusion. Data are presented as the mean ± SEM. Time measurements correspond to time of pump placement. *Differs ($P < 0.05$) from corresponding control mean.

manifold. Physical stress causes increased catecholamine secretion resulting in hyperglycemia (Bell, 1971). In addition, neurogenic amines such as adrenaline (epinephrine), noradrenaline, and glucocorticoids lead to increases in blood GLU by inducing the breakdown of glycogen to GLU in the liver in a variety of avian species (Bell, 1971; Assenmacher, 1973). Glucocorticoids have primary effects on metabolism, stimulating gluconeogenesis from proteins in muscle, lymphoid, and connective tissues. Stressed chicks normally exhibit elevated plasma levels of CS, whether treated with ACTH (Davis and Siopes, 1989), CS (Siegel and Van Kampen, 1984; Donker and Beuving, 1989), or feed restriction (Weber et al., 1990). Elevated blood levels of CS, in turn, cause increased energy levels by acting on intermediary metabolism of carbohydrates, protein, and fats. Corticosterone along with other blood-borne physiological variables is associated with ACTH-mediated gluconeogenesis from labile protein as indicated by an increase in nonprotein nitrogen concomitant with increased excretory uric acid level (Halliday et al., 1977; Siegel and Van Kampen, 1984).

The ACTH-treated birds exhibited higher levels of pCO_2, Hct, Hb, McHc, and HCO_3^- and lower levels of pO_2, K^+, Na^+, and Cl^- than control birds. In addition, there were no changes in pH and Ca^{2+}. Similar ranges of pH and blood gas values for chicken arterial blood as reported herein have been reported for fast-and slow-growing strains of broilers (Buys et al., 1999; Malan et al., 2003). Fetal arterial blood gas and acid-base status from intravenous infusion of ACTH in sheep also agree with our current results (Carter et al., 2002). Increases in Hct and Hb, along with reductions in pO_2, may be related to the increased metabolic activity needed to meet the energy demands for both maintenance and growth under

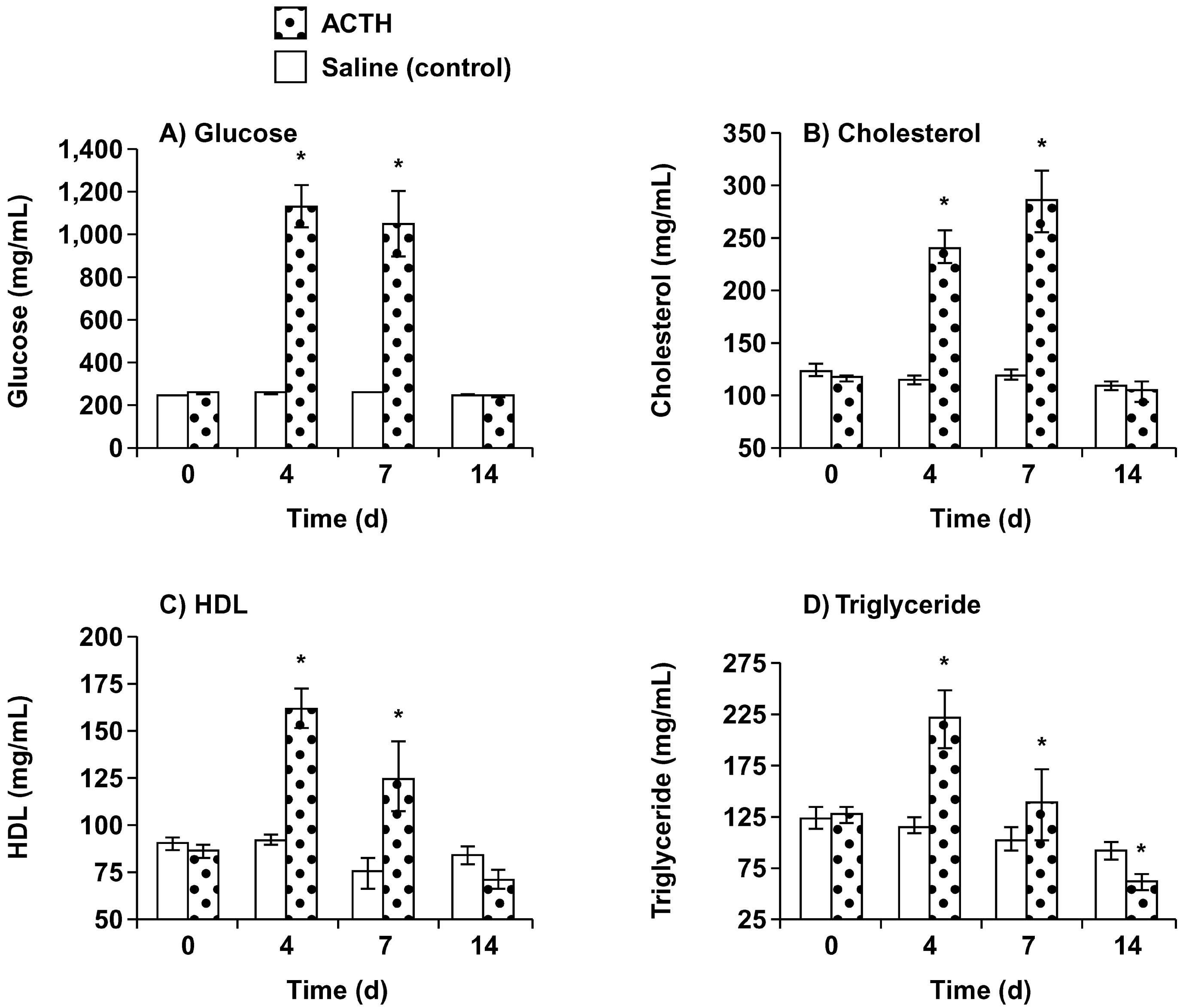

Figure 4. Circulating levels of glucose (A), cholesterol (B), high-density lipoprotein (HDL; C) and triglyceride (D) in broilers after adrenocorticotropic hormone (ACTH) infusion. Data are presented as the mean ± SEM. Time measurements correspond to time of pump placement. *Differs ($P < 0.05$) from corresponding control mean.

relatively extreme stressful conditions. Moreover, the increases in Hct, Hb, and McHc suggest increased erythropoiesis as a compensatory reaction to the lack of O_2 in the tissues.

The stimulation of adrenal steroid secretion by ACTH has been reported to cause an increase in plasma HCO_3^- in domestic fowl (Kutas et al., 1970). Programs of selection for high growth rate and muscle yield in broilers can have adverse effects upon structural, metabolic, and functional parameters in skeletal muscle, including spontaneous myopathy (Soike and Bergmann, 1998). These perturbations include reductions in systemic arterial pO_2 and saturation of Hb with O_2 (HbO_2) and increases in partial pressures of CO_2 leading to respiratory acidosis due to H^+ accumulation (Julian and Mirsalimi, 1992; Wideman and Tackett, 2000; Wideman et al., 2000, 2002). Plasma TRI levels increased in response to stress in this study. Whether this is an effect of epinephrine or another system responsive to stress remains to be tested. Epinephrine is known to increase TRI lipase activity (Norris, 1985), but glucocorticoids are known to inhibit synthesis of TRI from nonesterified fatty acids (Bentley, 1998; Remage-Healey and Romero, 2001). Nonetheless, because lipid appears to be the primary source of energy for most birds (Blem, 1990; Klasing, 1998), these results are intriguing and need further study. In addition, increased HDL and TRI levels at 4 and 7 d are in agreement with previous findings of Puvadolpirod and Thaxton (2000a,b,c,d).

The decrease in Na^+ concentration might be related to loss of Na^+ due to loss of water from the body that results when extracellular fluid volume is decreased. There was also a decrease in Cl^- concentration, which is interpreted to be secondary to the shift of Na^+ and K^+. Also, Cl^- ions are the most abundant of osmotically active solutes, and

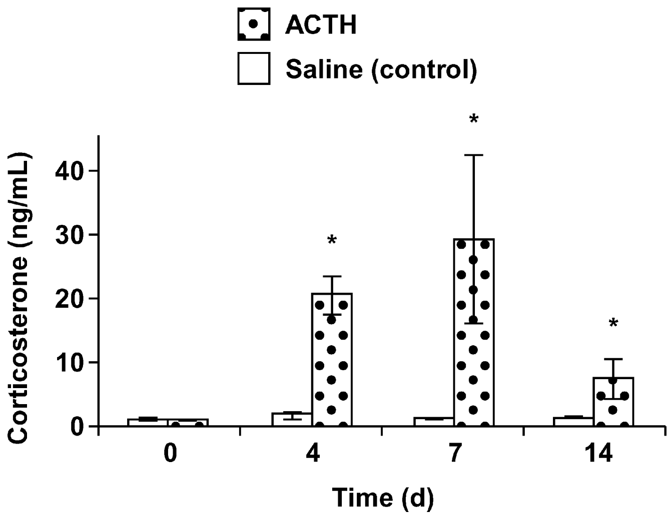

Figure 5. Circulating levels of corticosterone in broilers after adrenocorticotropic hormone (ACTH) infusion. Data are presented as the mean ± SEM. Time measurements correspond to time of pump placement. *Differs ($P < 0.05$) from corresponding control mean.

changes in Cl^- are to a great extent secondary to changes in Na^+. However, the amount of Na^+ in the extracellular fluid compartment is the most important factor. Based on the key position of Na^+ in volume homeostasis, more than one mechanism operates to control the excretion of the Na^+. Chickens infused with ACTH are known to exhibit polydipsia and polyuria throughout the stress and recovery periods (Puvadolpirod and Thaxton, 2000d), and there has to be excess water consumption to clear metabolic uric acid and excess electrolytes (Siegel and Van Kampen, 1984).

Erythropoietin production by kidney cells in chickens and its putative stimulatory influence on bone marrow production of erythroblasts is well known (Samurut and Nigon, 1976; Pain et al., 1991; Wickeramasinghe et al., 1994). Additionally, glucocorticoids have been shown to be required for sustained proliferation of erythroid progenitor cells (Bauer et al., 1999). Thus, our finding that Hct and Hb were elevated in the ACTH-treated birds suggests that erythropoiesis was activated. Since pO_2 concentration, as well as Hct and Hb, remained elevated on d 14 (i.e., 7 d after the infusion of ACTH was terminated) this erythropoietic adaptive change increased transport of O_2 to cells, and it may be a permanent adaptation. Additional research is required to determine if new set points for Hct and Hb have occurred or if these parameters will return to pre-ACTH values.

During stress, glucocorticoids activate gluconeogenesis. This involves conversion of noncarbohydrate moieties into GLU. The most common substrate for gluconeogenic conversion into GLU is amino acids. During metabolic production of GLU by both glycolysis and gluconeogenesis, each mole of ATP that is required for an enzymatic step utilizes 1 mol of O_2. Therefore, not only is gluconeogenesis costly from an energy production standpoint, it is equally costly in terms of O_2 use. It should be noted that gluconeogenesis can be sustained as long as the animal maintains a protein reserve (Klasing, 1998), has adequate supplies of O_2 (King, 2006), and does not deplete electrolyte reserves so that homeostatic acid-base balance is disrupted (Julian and Mirsalimi, 1992; Wideman and Tackett, 2000; Wideman et al., 2000, 2002).

Results of the present study indicate that chickens experiencing acute physiological stress resulting from continuous infusion of ACTH exhibited elevated CS levels, increased blood levels of the major metabolic substrates (i.e., GLU, CHOL, HDL, and TRI), and reduced levels of pO_2 concomitant to increased levels of pCO_2 and HCO_3^-. Additionally, acid-base balance was maintained by moment-to-moment changes in electrolytes to insure pH balance. These changes seem to be an essential part of stress because they insure that the energy needed to adapt to a stressful situation can be produced.

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